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A Study of *Ballota limbata* as an Alternative Medicine for Eye Diseases

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Abstract

Ballota limbata BTH. (syn. *Otostegia limbata* (BTH.) BOISS (Lamiaceae) is a medicinal herb used in folk medicine to cure a number of ailments. An aqueous extract of the herb is locally used for the treatment of eye inflammations and infections. To explore the possible cause of this application of the herb, we studied mineral elements present in it, as well as its antimicrobial effect against *Bacillus subtilis*. The leaves of *B. limbata* contain considerable amounts of zinc (4.6 mg/100g) and copper (0.847 mg/100g) which are known for their role in eye health. The quantity of iron (52.7 mg/100g) and calcium (1972 mg/100g) is also good, and the ratio of K (393.4 mg/100g) to Na (45 mg/100g) is extraordinarily high. This peculiarity of the herb may make it a very good remedy for hypertension. The leaves extracts also showed antimicrobial activity against the tested bacterium, which may explain the use of the herb against eye infections.

Keywords: *Ballota limbata*, eye diseases, mineral elements, *Bacillus subtilis.*

Introduction

Ballota limbata BTH. (syn. *Otostegia limbata* (BTH.) BOISS) belongs to genus *Ballota* (Lamiaceae) which consists of about 33 species mainly found in the Mediterranean region (Citoglu et. al., 1998). The species of genus *Ballota* are known for their therapeutic uses against various diseases. The leaves and the tops of *Ballota saxatilis* are used for colic, asthma, influenza, insomnia, and haemorrhoids. Infusions prepared from the leaves have been reported to possess antiulcer, antispasmodic, and sedative activities. Aerial parts, and their aqueous and hydroalcoholic extracts are widely used in European medicine for their neurosedative activities (Citoglu et. al., 1998). *Ballota larendana* and *Ballota nigra* have potent antidepressant activity, while *B. larendana* possesses anxiolytic activity

(Vural et. al., 1996), and its constituents have also been shown to possess antiulcer, antispasmodic, antidepressant, anxiolytic and sedative activities (Vural et. al., 1996). Methanolic extract of *Otostegia persica* has been found to exhibit strong antioxidant activity (Shrififar et. al., 2003).

In Pakistan, *Ballota limbata* is widely distributed in hilly areas of NWFP and Punjab. In the local language it is called *Bui.* In folk medicine, it is used as remedy for a number of purposes. It is well known that the aqueous extract of the leaves of the plant are used to treat ophthalimia by the local people (Nasir and Ali, 1972; Chopra et. al., 1956). The application is also confirmed by the number of interviews the present authors have conducted in the area. The roots and leaves of the herb have been reported to contain diterpenoids (Farooq *et. al*., 2007).

The present investigations were carried out to explore possible scientific base for the use of this *Ballota limbata* for the treatment of eye inflammations and infections, and reported here for the first time. With this in view, various trace elements present in the aerial parts of the herb have been determined to ascertain the reasons for the claimed efficacy of *Ballota limbata* against eye inflammations. Antimicrobial activity of extracts of the aerial parts of this plant in various solvents has also been determined to evaluate the use of this plant to cure eye infections.

Materials and Methods

Collection of Plant Material

The aerial parts of *Ballota limbata* (Lamiaceae) were collected in October 2007 from hills near Abbottabad (Pakistan), and identified by the taxonomist of Hazara University, Mansehra. The leaves of the herb were separated, dried under shade, and crushed and converted into powder form.

Trace Elements Determination

A weighed sample (5.0 g) was placed in a crucible and heated on flame for about 10 minutes to remove moisture and volatile matter. Then the crucible was heated in a furnace at 600 ˚C for about 4 hours which converted the sample into ash. The ash was dissolved in Conc. HNO_3 (12 mL) by adding the acid gradually. Then the total volume was made 100 mL by adding twice distilled water to the dissolved material. The content was then filtered to get a clear solution which was used for the analysis. The atomic absorption spectrophotometer used for trace elements analysis was Varian Model AA240 (AAS).The experiment was done in triplicate and the results were averaged (Table 1).

Antibacterial Study

The antibacterial activity of various extracts of the leaves of *B. limbata* in solvents of different

polarities including sterile water, ethanol, methanol, and hexane was carried out on *Bacillus subtilis* (ATCC 6633 BP/USP). A weighed sample (5.0 g) of the powdered leaves was transferred into a 250 mL round bottom flask containing 200 mL sterile water, and the extraction was carried out using Soxhlet apparatus for about 4 hours. The extract so obtained was filtered through Whatman filter paper no. 40 by using vacuum filtration assembly. Extracts in hexane, methanol, and 70% ethanol were obtained in the same manner: taking 5.0 g powdered leaves in 200 mL each of the above mentioned solvents, and extracting for 4 hours. All the four extracts were then concentrated to about 5 mL through evaporation under reduced pressure.

Antibacterial activity of the extracts was by using the method reported by Asghari *et. al.* (2006) with some modification. Briefly, the microorganism was suspended in sterile saline and diluted at 1.5 x 10^6 organisms per mL. It was inoculated onto the surface of Muller-Hinton agar plates, and left to dry. Six mm diameter wells were cut from the agar using a sterile cork borer. Then, 0.1 mL of a herbal extract was delivered into the wells. The cell cultures were refrigerated for an hour in order to allow the herbal extract spread. After incubation at 37 °C for 24 hr, plates were examined for any zones of growth inhibition. In case of each extract the corresponding solvent (sterile distilled water, ethanol (70%), hexane, or methanol) was used to serve as negative control. Each experiment was repeated five times and the inhibition zone diameters were examined. The results of the investigation are presented in Table 2.

Results and Discussion

The plants have provided human beings with medicinally useful substances since time immemorial and are the oldest, as well as the cheapest, source of pharmacologically active chemical compounds (Ncube, 2008; Prusti *et al.,* 2008). Quite a large number of remedies based on local herbs are being used particularly by people residing in rural areas. Serious efforts are, however, required to scientifically justify the use of medicinally important plants for mitigating the sufferings caused by very large number of sicknesses.

The herb *Ballota limbata* is therapeutically useful for a number of diseases including eye inflammations and infections. As per the information we gathered from the area of Abbottabad (Pakistan), the aqueous extract of the leaves of this herb is used for the treatment of eye inflammation and infection (fresh leaves are crushed and ground in a mortar and water is added to prepare the extract). The effectiveness of the herbal extract against eye diseases may be due to, *inter alia*, the minerals present in it as well as its antimicrobial activity. In order to explore reasons for its folk use to cure eye ailments, we studied mineral elements present in this plant, as well as tested extracts in various solvents against *Bacillus subtilis* to determine their antimicrobial activity.

Mineral Elements

Various trace elements studied in *B. limbata* include macronutrients (Na, K, Ca, Mg) and micronutrients (Fe, Mn, Zn, Cu, Ni, Pb, Cd) (Table 1). The important elements are discussed below (Table 1.).

Table 1: Trace Elements in the leaves of *B. limbata.*

	Elements	mg/100 g
1	Iron (Fe)	52.7
2	Nickel (Ni)	0.672
3	Copper (Cu)	0.847
4	Cadmium (Cd)	0.059
5	Zinc (Zn)	4.6
6	Manganese (Mn)	8.0
7	Potassium (K)	393.4
8	Sodium (Na)	45.0
9	Calcium (Ca)	1972
10	Magnesium (Mg)	37.2

Potassium and Calcium

B. limbata leaves contain considerably high amounts of K (393.4 mg/100g) and Ca (1972 mg/100g), while the amount of sodium is quite low (45 mg/100g). This may make this plant useful in controlling hypertension. The intake of a decoction of the leaves of this herb can assist a person to overcome the deficiency of K and thus can enhance its functional role (Scrimshaw and Young, 1976). The RDA (recommended daily allowance) for Ca is 800 mg for adults and 1200 mg for teenagers. *B. limbata* leaves can be used to make up the deficiency of Ca in needy persons.

Zinc and Copper

The leaves of *B. limbata* contain 4.6 mg/100g of Zn. It is an important trace element which is part of about 70 enzymes. The role of zinc in eye health is important. It has been shown to play an integral role in maintaining normal ocular function. Zinc is the most abundant trace element in the eye and its concentration is 15 times higher in the retina than in the circulation (Karcioglu, 1982).

The ocular manifestations of zinc deficiency include altered vision, electroretinograms, and oscillatory potentials, and, if the deficiency is severe, ultrastructural changes are detected in the retina and retinal

pigment epithelium. Zinc supplementation may alter the progression of some degenerative retinal diseases (Grahn *et. al.*, 2001). Zinc is also an antioxidant element (Konerirajapuram *et. al.*, 2004), and, therefore, can give protection against free radical damage.

Zinc ions have astringent and slightly antiseptic effect and are used for the treatment of eye diseases in forms such as zinc sulfate. The well known eye drops called Oculosan contains 0.2 mg of zinc sulfate per mL.

The leaves of *B. limbata* also contain Cu in good quantities (0.847 mg/100g). Copper also plays a vital role in retinal cell survival and is crucial for normal functioning of antioxidant enzymes (Wills *et. al.*, 2008).

Iron

The high amount of Fe (52.7 mg/100g) in this herb is also notable. Iron is a part of many enzymes and an important component of hemoglobin. The RDA requirement for an adult male and female is 10 mg and 18 mg respectively. The leaves of *B. limbata* can prove to be an important iron supplement as only 20-40 g of the leaves can provide the required quantity of iron to a person.

Antimicrobial Study

The market is full of a wide range of allopathic drugs to provide relief to patients suffering from infections. The synthetic antimicrobial agents, however, have their own limitations. Most importantly, the pathogenic agents soon develop resistance to them rendering them ineffective. This poses enormous challenge to find a way out. It is, however, well known that plants provide a diverse array of secondary metabolites, many of which have diversified biological activities.

Here the purpose was to determine the antimicrobial activity of the aqueous extract of *B. limbata*, which is used by the local people to cure eye diseases. For comparison, the extracts of the plant in ethanol, methanol and hexane have also been tested for antimicrobial activity. The extracts were obtained directly in different solvents using Soxhlet apparatus. The details are given in the experimental section. All the extracts showed antimicrobial activity against *Bacillus subtilis* (Table 2). Since this microorganism is generally used to assess the activity of commonly used antibiotic drugs, the test is significant.

Table 2. Antibacterial activity of various extracts of *B. limbata.*

Microorganism	Growth Inhibition

	Water	Methanol	Hexane	Ethanol
Bacillus subtilis	+ve	+ve	+ve	+ve

Conclusion

Ballota limbata leaves have good quantities of various essential mineral elements. They are good source of Zn and copper which play important role in maintaining normal eye functions as well as in combating eye inflammation and infection. The herb is also rich in iron, potassium and calcium and therefore an aqueous decoction of the herb can be used to cure conditions such as hypertension. The herb is also effective against *Bacillus subtilis*, the microorganism commonly used to test the efficacy of antibiotics. Although more data is required to draw any conclusive inference, the antimicrobial activity of the herb is established. We intend to expand our investigations in different directions to prove the efficacy of such medicinal products. In this connection, assistance and guidance from relevant scientific quarters will be employed to achieve the desired goals.

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